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EV0266

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**Evaluation of methods for enrichment of carbapenemase-producing *E. coli* in pork meat and cecal samples of porcine and bovine origin**

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**Objectives.**

The European Commission (EC) legislation (2013/652/EU) has decided to initiate a voluntary pan-European specific monitoring of carbapenemase producing *Escherichia coli* in meat and cecal samples of both porcine and bovine origin. The isolation method to be used should ensure high detection levels of *E. coli* producing carbapenemases previously found in animal production. Based on our experience of validating a wide range of methods covering different brands of commercial and non-commercial selective and indicative agars to detect these isolates in beef meat (ECCMID 2014, eP334), a narrower selection of methods were validated to detect these isolates in pork meat and bovine and porcine cecal samples.

**Methods.**

*E. coli* isolates producing the following ESBL/AmpC/carbapenemase were included in the study: CTX-M-1 (ESBL), CMY-2 (AmpC), VIM-1 and OXA-48. Bacterial suspensions were added (spiked) to the matrices in concentrations of 0.1 (only meat samples), 1, 10, 100 and 1000 CFU/gram matrix sample. Two different methods for pre-enrichment were considered: 1) MacConkey broth and 2) Peptone Buffered Water (PBW), both without addition of any antibiotic. Pre-enrichment in MacConkey was done by incubating overnight at 44°C and in PBW at 37°C followed by semi-quantitative plating and overnight incubation at 37°C on ChromID CARBA, ChromID OXA agar plates and ChromID-SMART (BioMerieux).

**Results.**

*E. coli* producing CXT-M-1 or CMY-2 did not grow on neither the CARBA nor the OXA or the SMART plates, regardless of the chosen pre-enrichment method. *E. coli* producing VIM-1 were detected on CARBA and SMART plates (but not on OXA plates) by both pre-enrichment methods except when porcine cecal samples were incubated in MacConkey broth (method 1). *E. coli* producing OXA-48 was not detected when spiked to porcine cecal samples and incubated in MacConkey broth (method 1) but was detected on OXA plates and SMART plates after pre-enrichment in PBW (method 2). In general, detection limits were between 100-1 CFU/gram, being higher for CARBA and OXA-plates than for SMART plates.

**Conclusions.**

Protocols to specifically select for carbapenemase producers in pork meat and cecal samples from pigs and cattle were developed. These include a pre-enrichment step without antibiotics in PBW and subsequent plating on selective ChromID CARBA and ChromID OXA agar plates (higher detection limit), followed by incubation at 37°C. This method was the only of the tested methods, which was able to detect production of VIM-1 (on CARBA plates) and production of OXA-48 (on OXA plates) from all tested matrices.